
Human Recombinant Neuropeptide S Receptor Isoform A Stable Cell Line
Cat. No.: M00338 **Version 07302020**

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I. INTRODUCTION

Catalog Number: M00338

Cell Line Name: CHO-K1/Gα15/NPS1a

Aliases: NPSR1; VRR1; GPRA

GenBank Accession Number: NM_207172 (no expressed tags)

Host Cell line: CHO-K1/Gα15

Culture Properties: Adherent

Quantity: Two vials of frozen cells (>1×10⁶ per vial)

Stability: Stable in culture for minimum of 16 passages

Application: Functional assay for NPS1a receptor (Calcium flux assay)

Freeze Medium: 45% culture medium, 45% FBS (Cat. #10099-141, Gibco), 10% DMSO (Cat. #D2650, Sigma)

Propagation Medium: Ham's F-12K (Kaighn's) (Cat. #21127-022, Gibco), 10% FBS, 200 µg/ml Zeocin (Cat. #R250-01, Life Technologies) and 100 µg/ml Hygromycin B (Cat. #10687010, Invitrogen)

Mycoplasma Status: Negative*

Storage: Liquid nitrogen immediately upon receipt

II. BACKGROUND

The neuropeptide S receptor isoform a (NPS1a) is G_{q/11} and G_s-coupled GPCRs expressed in the bronchial smooth muscle cells, basally in colon epithelium and in occasional basal keratinocytes in skin. An Asn¹⁰⁷->Ile mutation is significantly up-regulated in a mouse model of ovalbumen-induced lung inflammation supporting a role in the pathogenesis of asthma.

* The mycoplasma test was performed with MycoAlert™ PLUS Mycoplasma Detection Kit of Lonza.

III. REPRESENTATIVE DATA

Calcium flux assay:

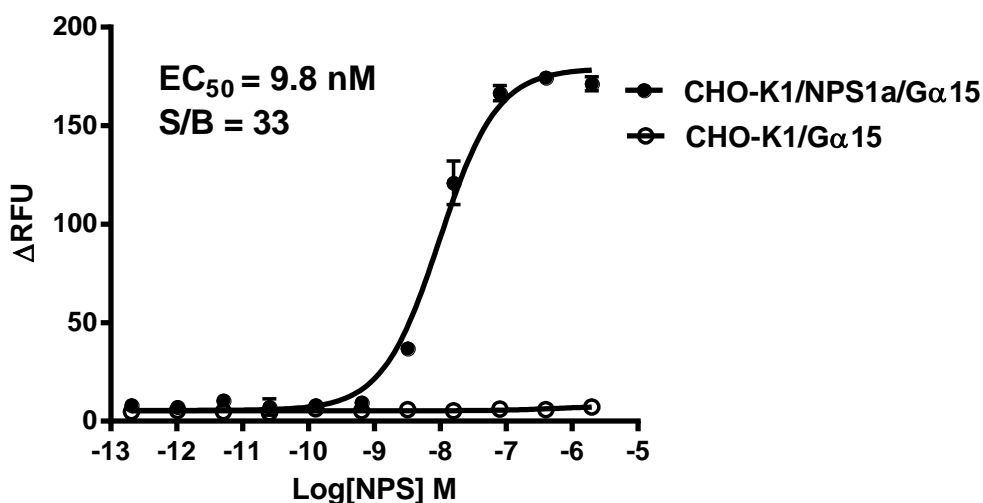


Figure 1. NPS-induced concentration-dependent stimulation of intracellular calcium mobilization in CHO-K1/NPS1a/Gα15 cells. The cells were loaded with Calcium-4 prior to being stimulated with agonist NPS. The intracellular calcium change was measured by FLIPR. The relative fluorescent units (RFU) were plotted against the log of the cumulative doses (5-fold dilution) of NPS (Mean ± SD, n = 2). The EC₅₀ of NPS on this cell was 9.8 nM.

Notes:

- EC₅₀ value is calculated with four parameter logistic equation:

$$Y = \text{Bottom} + \frac{(\text{Top} - \text{Bottom})}{(1 + 10^{((\text{LogEC}_{50} - X) * \text{HillSlope}))}}$$
 X is the logarithm of concentration.
 Y is RFU and starts at Bottom and goes to Top with a sigmoid shape.
- Signal to background Ratio (S/B) = Top/Bottom

IV. THAWING AND SUBCULTURING

Thawing Protocol

- Remove the vial from liquid nitrogen tank and thaw cells quickly in a 37°C water-bath.
- Just before the cells are completely thawed, decontaminate the outside of the vial with 70% ethanol and transfer the cells to a 15 ml centrifuge tube containing 9 ml of complete growth medium.
- Pellet cells by centrifugation at 200 x g force for 5 min, and remove the medium.
- Resuspend the cells in complete growth medium.
- Transfer the cell suspension to a 10 cm dish with 10 ml of complete growth medium.
- Grow the cells in incubator with 37°C, 5 %CO₂.
- In the following day, replace the cells with fresh medium contains antibiotic.

Sub-culturing Protocol

1. Remove the culture medium from cells.
2. Wash cells with PBS (pH=7.4) to remove all traces of serum that contains trypsin inhibitor.
3. Add 2.0 ml of 0.05% (w/v) Trypsin- EDTA (GIBCO, Cat No. 25300) solution into 10 cm dish and observe the cells under an inverted microscope until cell layer is dispersed (usually within 3 to 5 minutes).

Note: To avoid cells clumping, do not agitate the cells by hitting or shaking the dish while waiting for the cells detach. If cells are difficult to detach, please place the dish in 37°C incubator for ~2 min.

4. Add 6.0 to 8.0 ml of complete growth medium into dish and aspirate cells by gently pipetting.
5. Centrifuge the cells at 200 x g force for 5min, and remove the medium.
6. Resuspend the cells in culture medium and add the cells suspension to new culture dish.
7. Grow the cells in incubator with 37°C, 5 %CO₂.

Subcultivation Ratio: 1:3 to 1:8

Medium Renewal: Every 2 to 3 days

V. REFERENCES

1. Xu YL *et al.* (2004) Neuropeptide S: a neuropeptide promoting arousal and anxiolytic-like effects. *Neuron*. 43(4):487-97.
2. Laitinen T *et al.* (2004) Characterization of a common susceptibility locus for asthma-related traits. *Science*. 304(5668):300-4.

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